

SOME ASPECTS OF NORMAL DEVELOPMENT IN THE COLONIAL CILIATE ZOÖTHAMNIUM ALTERNANS

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INTRODUCTION

This is the first of a series of inquiries into some of the problems of organization and regulation in a colonial protozoan of a rather special type, *Zoöthamnium alternans*, whose cells collectively possess in some degree many of the attributes of an individual organism. In order to fix some standard by which to judge experimental results it was first necessary to review in detail the characteristic features of normal development in a large number of cases.

Although Claparède and Lachmann (1858) formulated a scheme of notation for cell lineage in this species, Fauré-Fremiet (1930) was the first to make a comprehensive study of its development. His primary concern was the demonstration of cytological mechanisms by which potentialities for growth and ciliospore formation were assorted to cells in characteristic positions on the colony. According to his conclusions this "cytological" factor determines the general pattern of development. His second objective, a study of time as a limiting growth factor, has no immediate bearing on the subject to be treated here. The quantitative aspects and time relations in development are to be incorporated in a subsequent report.

The data presented here augment in considerable detail the previous studies of *Zoöthamnium alternans*, particularly with regard to the features of development which have an important bearing on the problems of regulation now being investigated.

The work was done at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the summer months of 1935-6.

It is a privilege to acknowledge assistance from those whose friendly interest has sustained the author's enthusiasm over many of the dull periods of routine work.

MATERIAL

The basal portions of mature hydroids (*Pennaria* and *Tubularia*) and Bryozoa (*Bugula*) from many localities in Woods Hole harbor great numbers of attached colonies of *Zoöthamnium alternans*. The

hydroid and bryozoan colonies are clean and free from the ciliates until about June 15. From then on throughout the summer the protozoa are very abundant, although their condition varies with particular habitats and with the water temperature. Grave (1933) found that the hydroids of this region are particularly healthy and free from bacterial growths, debris, etc. for the early spring months but that in mid-summer the contaminating organisms affect them adversely. This applies equally to the associated protozoa, for those obtainable in late July and August are commonly infested with parasites of several classes. The most annoying and destructive organisms are bacteria, filamentous algae, and an intra-cellular parasite which has been identified as a suctorian (*Acineta*) by Fauré-Fremiet (1930); others of less consequence are small crustacea, diatoms, and other ciliates, e.g. *Amphileptus* and *Cothurnia*.

METHODS

The procedure for maintaining selected colonies in aquaria is an elaboration of the technique employed by Fauré-Fremiet (1930) for his study of growth in *Zoöthamnium alternans*. Hydroids bearing the protozoa were cleaned, washed, and packed into large glass vessels of running sea water. Ruled and numbered slides were placed face down on the infected hydroid material and left for about 12 hours. They were then removed and washed by passing under a stream of running water. The precautions taken at these stages to remove adhering scum, debris, algae, other protozoa, and minute crustacea, determined to a great extent the age to which the colonies could be grown.

Some of the mature asexual migratory cells, the ciliospores (Wesenberg-Lund, 1925), liberated from the "wild" colonies during the 12-hour interval, attached themselves to the ruled surface of the numbered slides. Slides were examined in Petri dishes, the positions of the recently affixed ciliospores recorded, and then transferred from the Petri dish to correspondingly numbered slots (vertical) in wooden racks, immersed in a 15-gallon aquarium. Cultural conditions were very much improved by the use of filtered sea water. Two 2-liter aspirator bottles packed with sand and stoppered with glass wool proved to be adequate for the purpose. When cleaned daily their combined outputs averaged about 50 gallons per hour.

Routine counts were made with a 4 mm. dry objective. When optical sections of the barely immersed colonies were not clear, the water level in the Petri dish was adjusted by a slight turn of the dish on the slanted microscope stage until the surface film flattened the

colony. This was done as a last resort, for contact with the surface film elicited rapid contractile responses, frequently resulting in the loss of important zoöids.

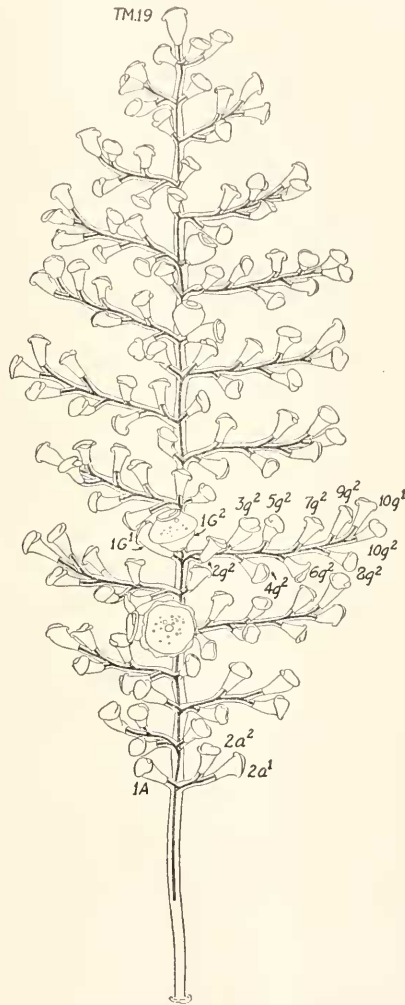


FIG. 1. A relatively mature colony of *Zoöthamnium alternans* showing the characteristic arrangement of the zoöids and branches. Axial microzoöids on branches *E*, *G*, *L*, and *N* are differentiating into ciliospores; one of them at *E* is in an advanced stage of metamorphosis, almost ready to break away from the parent colony. Approximately $\times 100$.

GENERAL FORM OF THE MATURE COLONY

The tapering frond-like configuration of the older expanded colonies is shown in Fig. 1. Its branches project obliquely from the per-

pendicular axis. They lie in the same plane but alternate on right and left sides at successive nodes. The zoöids of the branches occur singly for the most part, likewise alternating in position along the branch axis. While the branches of this species usually consist only of a single axis, in rare cases the zoöids may divide one or more times to produce branches of the second order.

The expanded colony is essentially asymmetrical, i.e. characterized by three heteropolar axes. The primary axis is firmly cemented to the substrate at the basal end and bears the actively dividing terminal macrozoöid (*T.M.*) at the apex. The lateral axis is heteropolar by virtue of the alternating branches, each one terminating in a single cell, the dominant branch zoöid. "Anterior" and "posterior" surfaces (Fauré-Fremiet, 1930) are defined by the curvature of the branches as well as by the position of the zoöids: the branches curve anteriorly while the zoöids are inclined posteriorly (toward the convex surface). A sample count showed that the first branch occurred nine times on the left side to four on the right. Both dextro- and leiotropic colonies were reported in another species, *Zoöthamnium arbuscula*, by Furssenko (1929).

Beginning at a point about mid-way between the basal (attached) end of the stalk and the first branch, a heavy contractile central cord, or "neuro-muscular" cord, within the stalk substance ramifies throughout the colony (Fig. 1). Its branches terminate distally at the basal ends of the zoöids. It is thus continuous from branch to branch and from cell to cell. More will be said about the importance of this structure in a later section.

NORMAL DEVELOPMENT

The scheme used to designate the lineage of the zoöids is a modified form of Fauré-Fremiet's adaptation of the original rule formulated by Claparède and Lachmann (1858-60), who were the first to note development in this species according to a definitely determined pattern.

The mode of propagation in *Zoöthamnium alternans* is almost exclusively asexual. Axillary branch zoöids at various levels in larger colonies are transformed into migratory ciliospores. These liberated motile individuals are comparatively large cells, metamorphosed zoöids, whose sub-conical bodies flatten antero-posteriorly into cells having a thickened bi-convex lens-like appearance. Their rapid swimming or creeping movements are effected by means of a heavy equatorial girdlet of cilia. The oral disc and peristomal cilia are retracted and probably undergo reorganization during the motile phase.

After a migratory existence of several hours duration the ciliospores become relatively quiescent, hovering about within a limited radius with aboral end in contact with the substrate, finally fixing themselves to the surface by means of a scopula. The secretion of the stalk begins; the girdlet of cilia becomes inactive and disappears as the peduncle elongates. The lens-shaped ciliospore then begins to assume the appearance of a very large sub-conical zoöid with retracted adoral zone. The first section of the peduncle secreted is a fairly heavy, non-contractile cylinder with thickened cuticle and homogeneous hyaline medullary substance. At some point between 200–300 μ the secretory activity of the ciliospore is altered: the continuity of the secretory process is interrupted, the basal end of the cell increases in diameter, and the formation of the neuro-muscular cord begins. This node marks the lower limit of stalk contractility. The contractile cord is an integral part of the stalk structure from this point onward.

The division of the initial individual occurs approximately 15 hours after attachment. The first division furrow defines the median plane of the future colony. Succeeding furrows bear a definite and constant relation to the first, in such a manner that the daughter cells occur first on one side of the mid-line then on the other. The two daughter cells resulting from the first division are always markedly unequal in size (Fig. 2). The large daughter remains apical (terminal) in position.

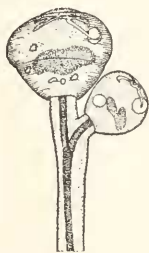


FIG. 2. Developing colony at the 2-cell stage. Drawn from a living specimen in which the macronuclei were faintly visible. $\times 250$.

In the current terminology it is designated as the terminal macrozoöid No. 1. The small lateral daughter, the first cell of the first branch, is characterized as the median microzoöid *A*.

Dichotomous branching of the stalk occurs when the secretory basal portion of the cell constricts during the fission process. With the onset of secretory activity (i.e. stalk formation) the sister cells gradually move away from the point of origin. Material secreted by the terminal macrozoöid prolongs the axial stalk. The branch cell produces a somewhat smaller stalk extending laterally from the junction.

The apical cell (*T.M.* No. 1) divides again at the second node in approximately 8 hours, producing an apical cell of the second generation (*T.M.* No. 2) and a median microzoöid *B*. This time, however, the branch cell lies on the opposite side of the axis from the previously produced microzoöid *A*. Repeated divisions of the apical cell extend the principal axis of the colony, each time similarly producing a terminal macrozoöid of the succeeding generation (3, 4, 5, . . .) and an alternating branch zoöid of a higher level (*C*, *D*, *E*, . . .). The position of successive branch microzoöids is strictly alternative. Colonies with as many as 25 branches were frequently observed on the culture slides but it is doubtful that this represents maximum development. One colony produced 33 branches before succumbing to external parasites.

Following the first division of the apical cell there is a gradual reduction in the size inequality of the resulting daughters until, after 8 to 10 generations of axial development, or in some cases even fewer, their volumes immediately after a mitotic period are approximately equal. For experimental purposes it is essential to distinguish between recently formed daughters of later generations along the main axis. To this end it is necessary to refer back to the previous generation. If the left-hand cell of that generation is differentiating into a branch cell, then the right-hand position of the undifferentiated cell at the new node may be taken as assurance of its presumptive branch relations. Likewise position is the chief diagnostic criterion for the identification of undifferentiated branch zoöids.

The generalized pattern of branch development given below concerns only the position of the cells produced, for as will be seen later, the descendants of the median microzoöids are not always equivalent in their prospective values. The symbol *X* (or *x*) is used to denote branch generations in general (Fig. 3).

Lateral extension of the stalk bearing the median microzoöid *X* continues until the cell divides. Microzoöid *X* gives rise to two structurally similar cells, microzoöids *1X* and *1x*. Microzoöid *1X* occupies a median position with respect to the main axis of the colony, whereas *1x* is the lateral member. Actually *1x* immediately assumes the terminal position on the branch axis, thus constituting the growing point of the branch. Marked differential behavior now characterizes the two sisters: *1X* lays down a short segment of stalk and then remains quiescent for some time; on the other hand, *1x* actively extends the branch axis, then divides again within a few hours. Microzoöid *1x* then produces *2x*¹ (median) and *2x*² (lateral). Cell *2x*² remains behind as a common nutritive branch zoöid while *2x*¹ assumes the terminal

position and continues as before, giving origin to $3x^1$ (lateral) and $3x^2$ (median); $3x^1$ to $4x^1$ and $4x^2$, etc. Each time the alternate secondary daughter is left behind as a common zoöid (Fig. 1).

Further mitotic activity in the common branch zoöids $2x^2$, $3x^2$, $4x^2$, etc. was not characteristic of the normal colonies whose histories were charted. About 2 per cent of the total number of colonies under surveillance showed a tendency toward precocious development. In these cases the repeated divisions of all branch zoöids led to such complex formations that the lineage soon became impossible to follow.

Conversely, microzoöid $1X$ on branches up to about level J commonly divides once, forming $1X^1$ (median) and $1X^2$ (lateral). Its mitotic activity on more distal branches is infrequent. On several

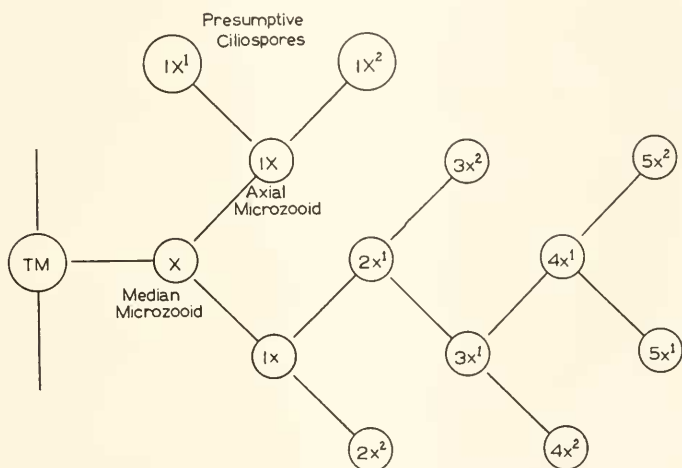


FIG. 3. Schematic representation of branch development.

branches of each colony the cell $1X$ or its descendants metamorphose into the migratory ciliospores which break away from the parent colony, leaving behind a short, stubby peduncle. Occasionally $1X$ produces a second or sometimes a third generation thereby augmenting the number of propagatory zoöids.

DIFFERENTIATION

The distribution of the heteromorphic zoöids to be described is a generalized account based upon a tabular evaluation of 200 cases of normal and experimentally cut colonies (proximal portions only), many of which were lost before complete development. Inclusion of the basal parts of operated colonies in the tabulations seems to be justified since statistical summaries of both normal and operated

colonies indicate no consistent variation as regards either rate of development or position of the differentiating zoöids.

During the life history of a colony six types of zoöids may be formed although not all of them are apt to be present at any one time.

1. The *common nutritive microzoöids* constitute the majority of the colony cells. They are the trumpet-shaped individuals characteristic of the genus. Notwithstanding the fact that most of them never assume any other form, they may be regarded as generalized components of prime colonies because (a) under undisturbed laboratory conditions some of them at random positions along the branch axis metamorphose into microgamonts, and (b) experiments may be performed in which variously situated common zoöids may be induced to differentiate into actively reproducing apical cells. Their capacity to differentiate into another type under imposed conditions gradually diminishes in time so that in older colonies the "vegetative" function becomes fixed. They can then be considered as somewhat specialized zoöids. The shape of zoöids that have persisted for relatively long periods of time without division are decidedly more slender and elongate than the "average" zoöids. This appears to be a modification of form rather than an alteration of volume. The extension of the protoplasmic body as well as its peduncle also appears to be gradual and continuous subsequent to the post-mitotic growth period.

2. Each colony bears a single *terminal macrozoöid* (TM.) at the apex of the principal axis (Figs. 1 and 2). In the earlier stages of colony formation three structural peculiarities clearly distinguish it from the common zoöids. It is considerably larger than any of the common types for the first ten or more axial generations, the body is characteristically flexed, and numerous annular striations in the pellicle are very pronounced. After ten generations or so its form individuality progressively diminishes. At levels beyond the twentieth branch only its position and generative activity are distinctive.

3. The *terminal branch zoöids* ($1x$, $2x^1$, $3x^1$, . . .) are almost diminutive replicas of the terminal macrozoöid. For the first few generations they are identified shortly after dividing by fairly marked pellicular annulations and a moderate anterior flexure of the tapering body. Near the close of branch development their patent features are obscure. Standard criteria of position and, to some extent, proclivity toward more rapid stalk formation and division must be relied upon to distinguish them from their common neighbors, the secondary daughters. Finally, at the terminus of a fully formed branch the last pair of zoöids are usually indistinguishable. This point represents about four generations on branch *A*, ten at intermediate levels (e.g. *II*), and progressively fewer after that (Fig. 1).

4. The most striking of the heteromorphic zoöids are the immature *ciliospores*, or asexual propagative cells which represent differentiated microzoöids of the order $1X$ or descendants. It is important for later discussion to note that the function of ciliospore formation is normally but not invariably restricted to these microzoöids. They have been observed to arise from microzoöids $5d^2$ and $6d^2$ in one instance, and $10h^1$ and $10h^2$ in another. Fauré-Fremiet (1930) found that the terminal macrozoöid was sometimes transformed into a migrating ciliospore but, unlike the usual ciliospore-forming cells, was unaccompanied by an endomictic reorganization.

Prior to ciliospore formation microzoöid $1X$ undergoes a profound growth in size. Very accentuated concentrically disposed pellicular furrows appear during the growth process. It is impossible to predict whether or not it will divide, for in a small percentage of the cases it is transformed directly into a motile ciliospore thereby terminating the lineage of that cell on the colony. Division of $1X$ in from 20 to 70 hours after derivation from the median microzoöid X is the rule, however. It is noteworthy, perhaps, that fission may occur after the onset of differentiation. The increased volume at this time is considerably in excess of the pre-divisional growth of the other zoöids. The two descendants, $1X^1$ (median) and $1X^2$ (lateral), are seldom equivalent in their size relations; the lateral daughter is usually the larger and the first to metamorphose.

Complete differentiation into motile cells already characterized involves the appearance of a girdle of long, closely set cilia about the equatorial region, the accentuation of the annulations, introversion of the oral disc, and differential growth in the plane of the diameter, eventually flattening the zoöids into disc-shaped bodies. It is believed that they are able to feed to some extent when the oral disc is introverted and the adoral zone folded. The gullet remains open and the undulating membrane within persists in its activity. The final metamorphosis of $1X^1$ may be deferred until a second generation of potential ciliospores ($1X^{11}$ and $1X^{12}$) are produced. The origin of at least four ciliospores from some axial microzoöids has been observed. Whether or not they represent three generations of alternate development or two generations formed dichotomously is still an open question.

The production of ciliospores is restricted to a limited number of loci on any given colony. Commonly there are but three or four such loci on one colony, although one protocol shows as many as seven. A generalized colony as visualized from tabulated data bears florescences at levels $D-K-P-T-AA$. They have been recorded for all nodes along

the axis between *B* to *W* inclusive, but never at consecutive nodes in any one case. It should also be stated that 1*X* on many branches divides without giving rise to ciliospores. Its descendants persist as common microzooids.

5. *Microgamonts* (*microgonidia*, Wesenberg-Lund, 1925; *microconjugants*, Furssenko, 1929) develop from otherwise visibly undifferentiated microzooids at the terminal or sub-terminal position along branch axes. The observed time interval between the formation of the microzooids and their complete transformation into mature, liberated swimmers varies between the limits of six to thirty-six hours. The first sign of approaching metamorphosis is the appearance of a faint furrow about the equatorial region of the tapered body. The zooid is thus marked into a larger peristomal portion and a smaller basal portion. Cilia gradually grow out from the body at the furrow. At first very short, they soon develop into a broad girdle whose movements simulate those of a slowly waving undulating membrane (Fig. 4). Meanwhile the diameter of the cell increases. The oral half



FIG. 4. Differentiating microgamont about 5 hours before the completion of metamorphosis. $\times 250$.

containing the retracted peristomal disc is transformed into the slightly flat, sub-conical oral end of the swimmer. The posterior portion is further reduced to a very slightly convex aboral surface at whose center the stalk is still attached. Its struggling movements gradually accelerate until at full maturity it breaks away from the parent colony. At full term the microgamonts resemble ciliospores in their eccentric body outline, ciliature, and general movements. They are, however, not perceptibly larger than the common zooids, show but faint indications of annulations, and swim more rapidly.

While cytological studies were not attempted, it is definitely established that these are the migratory members of the copulatory series. When liberated some have been observed to swim rapidly back and forth in an arc about the parent colony, occasionally coming to rest on some zooid, or crawling about on the branches and over the individuals in various positions, and eventually swimming away as though its relatives were not receptive of its attentions. The duration of the migratory phase was not determined. One was observed to settle upon terminal macrozooid No. 3 of a young colony possessing nine cells in all. Two and one-half hours later the aboral end of the

migrant was firmly fused with the lateral surface of the apical cell. By that time the ciliary crown of the swimmer had been resorbed.

As regards origin, it is frequently difficult to determine if microgamonts arise from prospective terminal or sub-terminal branch microzooids because of the facility with which the prospective values of very recently produced daughter cells may be altered. For example, if the terminal cell (e.g. $2x^1$) is accidentally lost, the sub-terminal cell ($2x^2$) immediately assumes the terminal functions. If loss occurs soon after fission, when both daughters are superficially alike, the stalk remnant left behind at the node occurs in the alternate position, whereas the cell which normally would have been the alternate ($2x^2$) becomes the growing point. The tendency of the terminal branch cell to differentiate into a microgamont is conclusively demonstrated by the successive metamorphosis of sister cells. This means that the continued lateral growth of a branch subsequent to the production of a microgamont may represent each time an early (undetected) transformation of the secondary zooid into a new terminal cell. The confusion is apt to arise because the alternate position of the stubby stalk left by the lost cell or by the migrating microgamont cannot always be diagnosed accurately. Fortunately in many instances the terminal branch cell is well indicated either by position or by symptoms of an approaching division by the time the alternate cell shows signs of becoming a microgamont.

Tabulations show that 85 per cent of the microgamonts observed on colonies whose complete developments were charted, were produced by the four basal branches *A*, *B*, *C*, and *D*, enumerated in decreasing order of importance. The rest arose from zooids on branches *E*, *F*, *G*, and *K*. They were formed either by the terminal ($-x^1$) or alternate ($-x^2$) cells as far out on the branches as the sixth generation on *A*, *B*, and *C* (including $5a^{21}$ and $5a^{22}$), the seventh generation on *D*, and the eighth generation on *F*. None were observed to come from the median microzooid *X*, the axial microzooid $1X$ or its descendants.

The majority of the colonies bore but one or two microgamonts; a few produced as many as nine but not more than three for any one branch. They occurred simultaneously on different branches but successively from the alternate zooids on any given branch. And finally, it is important that they differentiated only from branch zooids at or near the terminal position, never from the more axial (older) microzooids.

6. *Macrogamonts* were found only in the terminal macrozooid position on the primary axis. Attempts to discover some morphological or behavioral distinction between terminal macrozooids and pre-

conjugant macrogamonts were negative, but this possibility is not precluded since of the three or four hundred colonies inspected, none may have happened to have been preparing for conjugation. The presence of an attached microgamont constituted the sole criterion for identification.

Conjugants at all levels between the terminal macrozooid positions three to twenty-four inclusive were observed. The youngest colony possessed but nine cells in all, including the conjugant. Two successive conjugants, five generations apart, were recorded for one colony.

The incidence of conjugation reached a maximum during the month of July; before or after that the conjugants were quite rare. Even in July only about 5 per cent of the colonies bore conjugants although more of them were producing microgamonts. Many of the colonies produced no gamonts whatever during the period of development on the slides.

Some of the effects of conjugation upon colonial development will be treated in a later section.

REACTIVE CAPACITIES

The motor responses of *Zoöthamnium alternans* are developed to a relatively high degree. Very local reactions by individual zooids result in the retraction of the ciliated oral disc. More general responses evoked by altered salinity of the water, sudden contact stimuli, etc., are expressed by an extremely rapid contraction of the stalk and the zooids. Gradual relaxation or extension ensues within fifteen to thirty seconds unless the stimulus is sustained. This manifestation of irritability constitutes one of the major technical difficulties for experimental work. When the colony is contracted, it is practically impossible to distinguish a given zooid or to cut the stalk at a desired point. Fortunately for the experimental work the stalk alone may be touched gently without disturbing the colony. A series of attempts to inactivate the colonies by subjecting them to a variety of narcotics in various concentrations were quite unsuccessful. Freezing temperatures did not modify the contractions to any great extent.

SUMMARY

1. The frond-like colonies of *Zoöthamnium alternans* develop according to a well-determined pattern. This development is described with particular reference to the origin of the heteromorphic zooids.

2. An asexual propagative zooid (ciliospore) detaches from a mother colony, swims away, and settles down to form a new colony. When affixed to the substrate the ciliospore begins to secrete a peduncle or

stalk. The first portion of the peduncle is flexible but not contractile. From a point some 200 to 300 μ above the hold-fast, the ciliospore suddenly begins to produce a contractile cord in the core of the hyaline stalk substance. Distal to this point the contractile cord is an integral part of the stalk; it is continuous from branch to branch and from cell to cell.

3. When the stalk is approximately 500 μ long the ciliospore divides unequally. The larger daughter remains axial in position. It represents the first generation of the terminal macrozoöid series (T.M. No. 1). The smaller lateral cell is the initial zoöid of the first branch, the median microzoöid A.

4. Successive divisions of the terminal macrozoöid produce each time a terminal macrozoöid of the next generation and a median microzoöid. The latter are strictly alternate in position: they alternate on right and left sides of the primary axis at successive nodes. As many as thirty-three generations of the terminal macrozoöid have been observed.

5. The first division of the initial branch zoöid gives rise to an axial microzoöid and a lateral stem cell. The stem cell generates alternating zoöids of the main branch strain whereas the axial microzoöids on some of the branches represent the presumptive ciliospores.

6. At least four types of zoöids comprise a colony: (1) a single terminal macrozoöid at the apex of the primary axis, (2) many common microzoöids scattered along each branch axis, (3) a terminal zoöid at the tip of each branch, and (4) immature ciliospores which arise from axial microzoöids on some of the branches. During epidemics of conjugation two more types may be formed: the terminal macrozoöid may be transformed into a macrogamont, and common microzoöids at random positions may metamorphose into migratory microgamonts. The spatial distribution of the heteromorphic zoöids is described in some detail.

LITERATURE CITED

- CLAPARÈDE, E., AND J. LACHMANN, 1858-60. Études sur les Infusoires et les Rhizopods. *Mém. de l'Institut Genevois*, 5-7.
- FAURÉ-FREMIET, E., 1930. Growth and differentiation of the colonies of *Zoöthamnium alternans* (Clap. and Lachm.). *Biol. Bull.*, 58: 28.
- FURSSENKO, A., 1929. Lebenscyclus und Morphologie von *Zoöthamnium arbuscula* Ehrenberg. *Arch. f. Protist.*, 67: 376.
- GRAVE, B. H., 1933. Rate of growth, age at sexual maturity, and duration of life of certain sessile organisms, at Woods Hole, Massachusetts. *Biol. Bull.*, 65: 375.
- WESENBERG-LUND, C., 1925. Contributions to the biology of *Zoöthamnium geniculatum* Aryan. *D. Kgl. Danske. Vidensk. Selsk. Skr., naturv. og. math. Afd.*, 8. Raekke, X: 1.